inherent in the previously presented claim 1, that is, the act of recovery is inherent in the monomer yield recitation.

Rejection under 35 USC §112, first paragraph

Claims 1-3 and 5-15 are rejected under 35 USC §112, first paragraph, because the specification does not enable a person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The specification is allegedly deficient in teaching the rationale for choosing the specific pH values of the buffers used in the separations shown in the examples. According to the Office, the pH of the equilibration buffer appears to be an essential feature of the claimed methods, with reference to Arndt *et al.*, Biochemistry, 37: 12918-12926 (1998).

The prior art is said to provide no guidance in the selection of pH for mixtures of monomers, dimers, and multimers and whether pH is a factor that must be controlled in the selection of ion-exchange conditions for a method of separating monomers from dimers and multimers.

In view of the alleged lack of teachings in the specification for how to choose the appropriate pH of the equilibration buffer and lack of guidance in the prior art, the Examiner concludes that the specification fails to enable the full scope of the claimed methods, where the methods are used to separate the monomers from the dimers or multimers of any protein.

Applicants respectfully traverse this rejection. Enclosed is a Declaration by Philip Lester, a co-inventor in the above-identified application, addressing the various issues raised by the Examiner in this rejection.

Regarding how to choose the appropriate pH of the equilibration buffer, Philip Lester states in paragraph 3 that the skilled practitioner would select a pH based on the isoelectric point (pI) of the target protein. For instance, if the target protein has a pI of 7, one would select a pH of less than 7 for cation-exchange chromatography, and a pH of greater than 7 for anion-exchange chromatography. He notes that this is a starting point for all ion-exchange separations; one skilled in the art would routinely determine the pH at which the protein binds to make these separations and

could easily determine the stability of a protein at various pH levels as part of the consideration for purification, for example, by testing denaturation or activity loss. The present claims cover these acceptable pH ranges in specifying a pH of 4-7 or 6-9.

Philip Lester then cites a basic reference in support of this approach, i.e., the Pharmacia handbook, Ion Exchange Chromatography, Principles and Methods, 3rd edition, pp. 51-53 (April 1991), attached to the Declaration as Exhibit A. This reference shows that the prior art does in fact provide guidance in the selection of pH for mixtures of monomers, dimers, and multimers and discusses how pH and pI play a role in selecting ion-exchange conditions for the purification of these species.

Hence, determining the proper pH to effect the ion-exchange chromatography would not involve undue experimentation. Applicants have supplied enough information in the instant application to allow the skilled scientist to perform purification of polypeptide monomers away from their corresponding dimers and multimers as claimed, in the preferred embodiments and working example sections, and the pH ranges specified in the claims are not too broad.

Further, BSA and IgG, the proteins in the working examples of the present application, were carefully chosen as representative examples of proteins to use since they vary in molecular weight, pI, and structure. The molecular weight of BSA is about 68 kD, it has a relatively low pI (about 4.9), and it is a single-chain protein. In contrast, the molecular weight of IgG is about 150 kD, and it has a higher pI (about 8) and is composed of 4 subunits linked by disulfide bonds. The experiments with these two proteins show that the claimed purification method is operable with a broad range of proteins having different physio-chemical properties. See paragraph 4 of the Lester Declaration.

Arndt, cited by the Examiner as teaching that pH is a factor in the conversion of scFv monomers to dimers, describes a phenomenon of a protein that forms monomers or dimers depending on the pH and the ionic strength of the solution, as well as the presence or absence of antigen. The authors employ analytical size-exclusion chromatography (SEC) to determine the amount of monomer and dimer in their experiments, and they use SEC to separate monomer from dimer. SEC is a well established method for this type of

separation. Since Arndt does not even use ion-exchange chromatography to achieve separation, it is not applicable to the claims. In fact, Arndt supports the fact, disclosed in the background section of the present application on page 2, lines 2-3, that SEC was commonly used as of the filing date to separate monomers from multimers. See paragraph 5 of the Declaration.

The Examiner also cites Yang *et al.*, Mol. Immunol., 32: 873-881, especially bridging pages 876-877 (1995), as providing an example of the claimed method where ion-exchange chromatography was used for purification of scFv monomers, dimers and multimers, but was unsuccessful.

The Declaration addresses the Yang *et al.* article in paragraph 6. There is simply no indication in this paper that the authors were seeking to separate monomers from dimers and multimers using ion-exchange chromatography. Based on the information provided in Yang *et al.*, two conclusions can be reached: either (1) they did achieve separation of monomers, dimers, and trimers from each other during the ion-exchange chromatography but failed to recognize or appreciate this, and/or their pooling criteria were so broad that these forms were re-mixed into a single fraction, or (2) the monomer, dimers and trimers were aggregated together and therefore could not be separated during ion-exchange chromatography, and eluted as a single peak. In Philip Lester's opinion, the latter is the most likely to have occurred.

Yang *et al.* refer to a paper (Chaudary, V. *et al.*, (1989), Nature 339, 394-397), attached to the Declaration as Exhibit B, that describes a similar method from which Yang et *al.* derived their method. See paragraph 7 of the Lester Declaration. The Chaudary article describes the elution gradient in more detail, including slope, flow rate, fraction volume, etc. Chaudary claims to have separated monomers from "high-molecular weight aggregates," which eluted at higher ionic strength using ion-exchange chromatography (p. 395, col. 1). This is consistent with what others have reported in the literature, which is not what is claimed in the present application. Chaudary makes no mention of dimers, trimers, and multimers. In fact, the authors use a TSK 250 gel-filtration column to further purify the monomer--from what, they do not say--yet they say that "the chimaeric protein eluted

as a symmetrical peak at the location expected for a 65 K protein (data not shown)" (page 395, col. 1-2).

Philip Lester concludes one of two things from the results reported in Chaudary: either (1) there were no dimers, trimers, or multimers in the load, or (2) if these forms were present, Chaudary achieved only partial success in their separation and needed a second purification step, in this case SEC, to remove these forms. See paragraph 7 of his Declaration.

Addressing Yang *et al.* once more, Philip Lester states in paragraph 8 that several critical questions remain unanswered. One of them is what were they separating? It appears that they were separating the FV/TNF from host cell proteins. Indeed, the results presented in Figure 2 show that the described ion-exchange chromatography yielded a mixture of monomers, dimers, and trimers. The authors go on to state that the trimer form is the "active functional form" (*see* p. 878, col. 2, top, last sentence). They also imply that the other forms are active, suggesting that they were not attempting to purify desired monomers from such trimers, as claimed in the present application. Thus, Yang *et al.* report successful purification of a mixture of monomers, dimers, and trimers from unrelated bacterial proteins. In view of the state of the literature at that time (1995), they would likely have used SEC to purify the monomer from its dimers and trimers if they desired to obtain such separation. See paragraph 8(a) of the Declaration.

As mentioned in paragraph 6 of the Declaration as the second possibility, the feedstock may have contained only aggregate that would elute as a single peak: i.e., the feedstock could have contained no monomer, dimer, or multimer, but only higher-order aggregates. The only analytical method used to determine the x-mer forms was SDS-PAGE. Non-reduced SDS-PAGE can disrupt all non-disulfide-linked aggregates, and reduced SDS-PAGE can reduce all disulfide-linked aggregates. This could lead to results similar to those shown in Figure 2, gels A and B. Analytical SEC could answer this question, but it was not used in Yang *et al.* See paragraph 8(b) of the Declaration.

In conclusion, since Arndt has no relevance to separation or purification conditions, Yang is completely ambiguous as to what was separated and certainly the authors had no appreciation for separation of monomers, dimers, and multimers from each other, and the working examples herein show purification using representative proteins with a broad array of

properties, one skilled in the art would have sufficient information to have been able, as of the filing date of the present application, to perform successfully the purification method using the polypeptides and conditions as currently claimed. See paragraph 9 of the Lester Declaration.

The application herein satisfies the Ex parte Forman and In re Wand tests that consider certain factors in determining whether undue experimentation would be required. In particular, the quantity of experimentation necessary is not great, since the prior art, as exemplified by Exhibit A, shows how to use pI to select the proper pH. The specification provides an ample amount of guidance, especially in view of the two representative proteins provided and the level of skill in the art on ion-exchange chromatography as set forth in the accompanying Declaration. Two working examples are provided that show proteins with very diverse physico-chemical properties. The nature of the invention involves ion-exchange chromatography, which technique is widely known, including what pH to select as noted above. The prior art, as established above, does not clearly show unsuccessful or unpredictable results; the skill in the art of separation science is not extraordinarily high; and the claims are not overly broad, in that the pH ranges set forth are those that would ordinarily be used in ion-exchange chromatography depending on the pI of the protein, as evidenced by Exhibit A of the Declaration.

In view of the foregoing discussion and the Declaration establishing enablement of the claimed invention, applicants respectfully request reconsideration and withdrawal of the rejection under 35 USC §112, first paragraph.

Rejection under 35 USC §112, second paragraph

Claims 1-15 are rejected under 35 USC §112, second paragraph, as being indefinite for failing to point out particularly and claim distinctly the subject matter that applicant regards as the invention. The Examiner alleges that claim 1 is indefinite for lacking a recitation of an active step for recovering the monomer. Since claim 1, upon which all other claims ultimately depend, now specifies such active step to recite an inherency in a claim that was already definite, applicants respectfully request reconsideration and withdrawal of this rejection.

It is believed that the claims presented on the appended page are in condition for allowance based on the foregoing submissions. If the Examiner has any questions regarding this response, she is invited to call the undersigned.

Respectfully submitted,
GENENTECH, INC.

Date: February 19, 2003

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